Standard Operating Procedures for Analysis of TNT and RDX in Groundwater Using Colorimetric Method

The following standard operating procedure (SOP) was developed by the Army Corps of Engineers for use at the Umatilla Chemical Depot. It is repeated below as it appeared in their site specific Sampling and Analysis Plan.

Description

This SOP describes a field analytical method for determining TNT and RDX concentrations in water. The method uses solid phase extraction to remove and pre-concentrate the analytes from water. In the method, a 2 L water sample is passed through a stack of two membranes to pre-concentrate TNT on the top disk and RDX on the bottom disk. Acetone is used to elute RDX from the bottom disk, and a chemical reaction is induced that causes a color change indicative of RDX in the solution. The RDX concentration is estimated from the absorbence at 510 nm on a Hach DR2000 spectrophotometer. Next, the top disk is eluted with acetone and a different chemical reaction is induced causing a color change indicative of TNT. The TNT concentration is estimated from the absorbence at 540 nm on the Hach DR2000. The contract required detection limit for TNT is 1.0 µg/L and for RDX is 5 µg/L. Sample extraction and analysis may take between 1.5 and 5 hours per sample depending on the number of parallel extraction apparatus.

Safety Precautions

- Extraction and analysis should be performed in a well ventilated area.
- Laboratory technicians should wear chemical resistant gloves and safety glasses.

Extraction Procedure

Materials Needed (per sample)

2 Empore extraction membranes
aluminum foil
2 25x200 mm glass test tubes
filter flask apparatus
vacuum pump
tweezers
timer (minutes/seconds)

Reagents Needed (per sample)

2 L of sample
acetone, technical grade
DI water
tap water/DI water/acetone for cleaning

Pitfalls

- Never let the disks go dry. Throw the disks out and start over if they do. Keep the disks covered with at least ¼ inch of fluid during the extraction phase.

- Apply the vacuum gradually so as not to damage the membranes. If you see particles in the acetone extracts at this point, vacuum was applied too suddenly.

- Do not shake the sample prior to filtering.

Procedure

1. Use gloves during the entire procedure.

2. Use tweezers to place two Empore extraction membranes centered on the lower portion of the filter apparatus; cover squarely with the upper portion of the filter apparatus and clamp securely. Do not touch the membranes with your hands. A glass fiber filter may also be used to remove particulate.

3. Slowly add 30 mL acetone to the stack and allow it to soak for 10 minutes.

4. Slowly apply vacuum to the filter flask apparatus until there is minimum dripping of acetone (evidence that both filters are completely saturated). Shut off vacuum; add 10 mL of D. I. water. Let set for 10 minutes or until about ¼ inch of liquid remains, whichever occurs first. The next two steps go quickly, so have materials measured and in place before starting.

5. Fill the reservoir with sample before the fluid level is reduced to ¼ inch. Reapply vacuum ever so slightly. The sample may be filtered through at a rate of 10 to 100 mL/min.

6. Continue filling the reservoir until 2 L of sample has penetrated the membrane. Do not allow the fluid level to fall below ¼ inch until the entire sample has been passed through.

7. Add 10 mL of DI water to the reservoir just before the last of the sample penetrates into the membrane. This will aid in washing out the nitrate interference.

8. Continue to apply vacuum for about 2 minutes after the last of the sample has been extracted. This is to remove excess water.

9. Remove the upper portion of the filter apparatus from the filter stack and discard the glass fiber filter, if used.

10. Remove both the disks and set them face up on a clean piece of aluminum foil marked "T" for top disk and "B" for bottom disk, these will be used later for your TNT and RDX extracts.

11. Reassemble the filter apparatus and rinse first with DI water and second with acetone.

12. Disassemble the filter apparatus and pour the water from the 2,000 mL Pyrex flask into a waste container.

13. Wash a 25 x 200 mm tube with DI water, rinse with acetone, label the tube (RDX or TNT, sample number, date), place it in the flask, and replace the funnel.

14. Place the RDX disk membrane (bottom) on top of the lower portion of the filter apparatus. Reassemble the filter stack.

15. Add 7 mL of acetone to the reservoir and soak for exactly 3 minutes.

16. Apply vacuum and aspirate acetone into the 25x200 mm tube until dripping stops.

17. Remove the membrane and discard. Cap the 25 x 200 mm test tube. If possible, samples should be analyzed on the day of extraction. Otherwise, the meniscus should be marked on the test tube and the tube refrigerated. If the fluid level falls below the meniscus line, the tube should be refilled with acetone to its original level.

18. Reassemble the vacuum apparatus with the TNT (top) disk, which was set aside in Step 10 and a fresh 25 x 200 mm test tube (washed as described in Step 13).

19. Add 25 mL acetone to the reservoir and allow soaking for exactly 3 minutes.

20. Apply vacuum and aspirate into the 25 x 200 mm tube. Cap the 25 x 200 mm test tube. If possible, samples should be analyzed on the day of extraction. Otherwise, the meniscus should be marked on the test tube and the tube refrigerated. If the fluid level falls below the meniscus line, the tube should be refilled with acetone to its original level.

21. Decontaminate the reservoir and filter holder by washing with tap water, rinsing with DI water, and final rinsing with acetone.

22. Collect all liquids generated during the decontamination process for incorporation into the treatment plant process.

RDX Analysis

Materials Needed (per sample)

10 mL syringe with 0.45 µm filter
2 13 mL holding vials
30 mL syringe with 0.45 µm filter
desiccator and desiccants
Alumina-A filter
2 matched Hach cuvettes/stoppers
Hach DR2000 set to 510 nm
5 mL syringe with 0.45 µm filter
50 mL reaction vial
analytical balance
Kimwipes™
spatula
Miscellaneous glass volumetric pipettes, flasks, and graduated cylinders

Reagents Needed (per sample)

5 mL acetone, technical grade
Hach NitriVer 3 powder pillow
20 mL DI water
0.2 g of zinc dust, 100 to 325 mesh
RDX Standards for laboratory control
Miscellaneous amounts of tap water/DI water/acetone for cleaning

0.75 mL of acetic acid solution (77 percent glacial acetic acid and 23 percent DI water)

Pitfalls

- The reaction of the acidified extract with zinc is the most crucial step in obtaining consistent and correct results. The step should be done as quickly as possible (10 seconds at the longest). The reaction is also temperature dependent and should be performed in a cool setting. If the extract was refrigerated, make sure the extract is between 60-80 °F before beginning the analysis.

- The zinc syringe should be tapped gently so that the zinc is at the bottom of the syringe before removing the plunger.

- Check the filters at the bottom of the syringes to make sure that they are securely fastened before adding extract.

- Some samples may display a milky or cloudy appearance even after being filtered into the sample cuvette. These samples should be re-filtered and the cuvette cleaned. If the extract is still cloudy, read and record the absorbence, make a note of the cloudiness in the laboratory log, and indicate that this is a false positive. If a pink color also is present, this should be taken as a positive reaction for RDX; however, the associated result will be biased high.

- Make sure that the NitriVer pillow is completely dissolved in the reaction vessel containing 20 mL water. Do not let this solution sit for more than 10 minutes before using.

- Be sure to record the volumes used for all dilutions, not just the dilution factor. This will aid in checking for any mathematical errors.

- Let the bubbles dissolve before reading the absorption.

- Store the zinc dust and prepared zinc syringes in the desiccator.

- The test also will show a positive reaction for HMX.

Preparation Before Analysis

Using the spatula, place approximately 0.2 g of zinc dust into the barrel of a 5 ml syringe with a 0.45 μm filter attached. Replace the plunger. Store all zinc syringes in a desiccator with desiccant for at least 24 hours before they are used.

Procedure

1. Condition the alumina-A filter with 5 mL of acetone. Pour 5 mL of acetone into the 10 mL syringe with the alumina-A filter. Let the acetone filter through at a rate of one mL per minute.

2. Shake the 10 mL syringe dry and reuse for the next step.

3. Pour 5 mL of extract into the 10 mL syringe with the alumina-A filter. Filter the extract into a labeled 13 mL holding vial. Filter at a rate of 1 mL per minute.

4. Pour 5 mL of extract into the 10 mL syringe with attached filter. Filter the extract into a labeled 13 mL holding vial. Reserve the remaining 1 mL extract for possible dilutions.

5. Add 0.75 mL of the acetic acid solution to each 13 mL holding vial. Shake to mix and set aside for several minutes.

6. Add 20 mL DI water to a 50 mL reaction vessel. If the reaction vessel came supplied with DI water, remove the supplied water before adding fresh DI water. Add the NitriVer pillow to the 50 mL reaction vessel. Shake until completely dissolved. If batching samples, be sure to label the reaction vessel. Let set for at least 5 minutes but no longer than 10 minutes.

7. Slowly remove the plunger of the 5 mL zinc syringe, shaking the powder down. Holding the syringe over the reaction vessel, pour the extract into the 5 mL zinc syringe. Replace plunger, invert once and filter rapidly into the 50 mL reaction vessel containing 20 mL DI water. This step must be done as quickly as possible, approximately within 10 seconds.

8. Shake the reaction vessel to mix and wait at least 10 minutes, but no longer than 15 minutes, for color to develop.

9. Filter the sample into a clean DR2000 cuvette. Note in the laboratory logbook any obvious color.

10. Zero the instrument and obtain a background absorbence. (see Operation of Hach DR2000)

11. Read the absorbence of the sample and record along with any color changes.

12. Between samples, clean the cuvettes with DI water and acetone (in that order) using a stopper and shaking vigorously.

13. Periodically check that the instrument is correctly reading zero with the reference cuvette.

14. Calculate the concentration of the extract using the following equation:

$$RDX\ (\mu g/L) = Ai \times DF \times VCF \times RF$$

where

Ai = (absorbence of sample - absorbence of blank)
DF = dilution factor
VCF = volume correction factor is equal to 1.4 when the extraction volume is 7 mL
RF = response factor is listed in the laboratory

For sample concentrations where the absorbance is greater than 0.800, the reserved sample extract should be diluted with acetone, taken through the reaction steps, and the absorbance read and recorded.

TNT Analysis

Materials Needed (per sample)

30 mL syringe with 0.45 µm filter attached
Hach DR2000 set at 540 nm
2 matched Hach cuvettes/stoppers
Miscellaneous glass volumetric pipettes, flasks, and graduated cylinders

Reagents Needed (per sample)

Developer solution
DI water/acetone for rinsing
TNT standard for laboratory control

Pitfalls

- The test will also react for TNB and DNT.
- If the extract was refrigerated make sure the extract is between 60-80 °F before beginning the analysis.

Procedure

1. Zero the instrument and obtain a background absorbence. (see Operation of Hach DR2000)
2. Pour 25 mL of extract into a 30 mL syringe with attached filter. Filter the sample into the sample cuvette.
3. Read and record the initial absorbence.
4. Add one drop of EnSys TNT developer solution.
5. Shake tube continuously for 3 seconds.
6. Read the final absorbence and record. Also note any color present in the extract and how the color developed.
7. Periodically check the instrument is correctly reading zero with the reference cuvette.
8. Calculate the concentration using the following equation:

$$\text{TNT } (\mu g/L) = [Af - (2 \times Ai)] \times DF \times VCF \times RF$$

where

Ai = initial absorbence
Af = final absorbence
DF = Dilution factor
VCF = volume correction factor equal to 1.25 for 25 mL extraction volume
RF = response factor listed in the laboratory

Samples with TNT final absorbencies grater than 0.800 require dilutions. Use the reserved sample extract, perform the analysis, and record the results.

Quality Control/Quality Assurance

A laboratory control standard should be analyzed each day that an analysis is performed, and is used to verify that the analysis portion of the procedure is performed acceptably. The absorbence must be within 0.307 to 0.373 for RDX and 0.174 to 0.272 for TNT for the test to be in control. If the standard is not in control, try again, paying particular attention to the zinc step.

A blank must be extracted each day that samples are extracted. The method blank and its associated samples should all be analyzed at the same time. The blank must be clean and colorless. If any contamination is noted, review the glassware cleaning procedures or possible sources of cross contamination. Note problem and resolution in the logbook.

A blank spike must be extracted each week that samples are extracted. This blank spike is used to verify that the extraction portion of the procedure is being performed in an acceptable manner. A 2 L portion of DI water should be spiked with RDX and/or TNT and carried through the extraction procedure. Spike in 80 µL of a standard solution in acetone containing RDX and/or TNT at 500mg/L each. The concentration of standard in the final extracts will be 20 µg/L. The blank spike and its associated samples should be analyzed at the same time. The acceptable range for spike recovery is 60 to140 percent.

Field duplicates must be extracted and analyzed at a rate of 10 percent. The precision goal is ±50 percent RPD. Select duplicates that represent various concentration levels.

The reliability of the method is operator dependent. Each operator needs to do five qualifying spike samples through the extraction and analysis procedures to produce their own response factors for TNT and RDX analysis. The response factors need to be reevaluated periodically or when a major change in the procedure occurs.

All results and comments should be recorded in ink in a laboratory notebook with the name of the analysis and date clearly entered.

Operation of Hach DR2000

1. Turn on the Hach. The instrument will read “Selftest” followed by “Method?” Select “0” and press “read/enter”.

2. Rotate the wavelength dial to the desired setting: 510 for RDX and 540 for TNT. Approach the wavelength from the high side when adjusting.

3. Fill both cuvettes to the line with acetone.

4. Insert the “reference” cuvette into the cell holder with the side marked “25 mL” on the right.

5. Close the light shield and press “Clear/Zero” to establish the reference.

6. Remove the reference and place the “sample” cuvette in the holder with the side marked “25 mL” on the right.

7. Press “Read/Enter” and record the absorbence in the laboratory logbook as ABS background.

8. If the reading is greater than ±0.002, clean the cuvettes and repeat the procedure.

9. Proceed with sample analysis.

Cleaning Cuvettes

1. Fill the matched cuvettes with 5 mL of water.

2. Cap each cuvette and shake vigorously for 3 seconds.

3. Empty into a waste container.

4. Fill the cuvettes with 5 mL of acetone.

5. Cap and shake for 3 seconds.

6. Empty into waste container.

7. Repeat the acetone wash.

8. Wipe the outside of the cuvettes with Kimwipes™. Take care especially to clean the side labeled “25 mL” and the side opposite.

General Interferences

1. Do not use the reagents beyond the expiration date.

2. TNT samples must be analyzed immediately after adding the Developer Solution. RDX samples must be analyzed within 60 minutes of the color incubation step.

3. Operate test kits at less than 39 °C (100 °F). Store at less than 80 °F and out of direct sunlight.

4. Store all standards in the refrigerator.

References

Jenkins, T.F., P.G. Thorne, M.E. Walsh. Field Screening Method for TNT and RDX in Groundwater. U.S. Army Corps of Engineers, CRREL *Special Report 94-14*, May 1994.

Ensys. TNT Soil Test System, Strategic Diagnostics Inc., Newark, DE.

Ensys. RDX Soil Test System, Strategic Diagnostics Inc., Newark, DE.

U.S. EPA, *Explosives in Water Field Screening Technologies UMDA and SUBASE Bangor* (draft), prepared by Black and Veatch Special Projects Corp., Tacoma, WA, for U.S. Environmental Protection Agency Region 10, Project Number 71370, March 1996.